UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

011890

CASWELL FILE

APR 12 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorpicrin: Two year chronic gavage study. MRID 43744301. D218349. Tox. Chem. No. 214.

TO: Larry Schnaubelt/Susan Jennings (PM 72)
Special Review and Reregistration Division (7508W)

FROM: Stanley B. Gross, PhD, DABT, CIH
Toxicologist/Hygienist
Toxicology Branch I
Health Effects Division (7509C)

THRU: Joycelyn E. Stewart, PhD
Head, Section II, Toxicology Branch I
Health Effects Division (7509C)

I. SUBMISSION:

The following study was submitted to OPP to fulfill the chronic rodent toxicity study (GLN 83-1) requirement:

CITATION: Slauter, R.W. (1995) Two year oral (gavage) chronic toxicity study of chloropicrin in rats. International Research and Development Corporation (IRDC), Mattawan, MI. Laboratory Project Identification 656-003, June 19, 1995. MRID 43744301. Unpublished.

SPONSOR: The Chlorcpicrin Manufacturers Task Force, c/o Stephen Wilhelm, Niklor Chemical Company, 2060 E. 228th Street, Long Beach, CA 90810.

II. CONCLUSION:

The study is ACCEPTABLE.

III. STUDY SUMMARY.

The following summary is taken from the contractor DER (attached) prepared by Carol S. Forsyth, Oak Ridge National Laboratory, Task Order No. 95-17:

EXECUTIVE SUMMARY: In a two-year chronic toxicity study (MRID 43744301), chloropicrin (99%a.i.) was administered to groups of 30 male and 30 female Sprague-Dawley Crl: CD® BR, VAF/Plus rats by gavage at dose levels of 0, 0.1, 1.0, and 10 mg/kg/day for 104 weeks.

The only clinical toxicity observed was increased salivation immediately after dosing in males and females receiving 10 mg/kg/day. This finding was observed in 4-56% of each sex and persisted 15-30 minutes. At terminal sacrifice, survival rates in the 0, 0.1, 1.0, and 10 mg/kg/day groups were 53%, 43%, 37%, and 50%, respectively for males and 40%, 50%, 53%, and 30%, respectively for females. Body weights of males in the high-dose group were lower (not significant) than controls beginning at week 30 and continuing until the end of the study. Final body weights of the 1.0 and 10 mg/kg/day males were 88% of the control group mean (not statistically significant). Among females, the 10 mg/kg/day group had a significantly ($p \leq 0.05$) greater mean body weight as compared to controls at week 4 but no other differences were noted. There were no differences between treated and control groups of either sex for food consumption, urinalysis, hematology parameters, or organ weights at sacrifice. Several clinical chemistry values were sporadically significantly different from the control value but there were no dose- or treatment-related trends. At necropsy, there was a dose-related increase in the incidence of subcutaneous masses of the skin in females: 14, 19, 24 ($p \leq 0.01$), and 29 ($p \leq 0.01$) for the 0, 0.1, 1.0, and 10 mg/kg/day groups, respectively. Hyperkeratosis of the nonglandular stomach was seen in 7/30, 9/30, 11/30, and 20/30 ($p \leq 0.01$) males and in 6/30, 5/30, 11/30, and 24/30 ($p \leq 0.01$) females, respectively. Hyperplasia of the nonglandular stomach was observed in 3/30, 5/30, 4/30, and 18/30 ($p \leq 0.01$) males and in 6/30, 5/30, 6/30, and 14/30 ($p \leq 0.05$) females, respectively. The incidence of inflammation of the stomach was significantly ($p \leq 0.05$) increased in high-dose females (5/30 vs. 0/30 controls) but, while increased in a dose-related manner in males (1/30, 1/30, 2/30, and 6/30), did not reach statistical significance. Periportal hepatocyte vacuolation occurred in a significantly greater number of females in the 1 mg/kg/day ($p \leq 0.05$) and 10 mg/kg/day ($p \leq 0.01$) groups: 2/30, 6/30, 10/30, and 13/30. Hepatocyte vacuolation was significant in males only in the 0.1 mg/kg/day group (2/30, 8/30, 3/30, and 6/30). Females had a dose-related increase in the incidence of fibroadenoma of the mammary gland with statistical significance reached in the high-dose group ($p \leq 0.05$); incidence rates were 6/30, 9/30, 12/30, and 14/30 affected in the 0, 0.1, 1.0, and 10 mg/kg/day groups, respectively. High-dose females also had an increase ($p \leq 0.01$) in the rate of C-cell hyperplasia of the thyroid (23/30 vs. 13/30 controls). **The LOEL is 1.0 mg/kg/day, based on subcutaneous masses and hepatocyte periportal vacuolation in female rats and reduced body weights in males. The NOEL is 0.1 mg/kg/day.**

This chronic toxicity study in the rat is acceptable and does satisfy the guideline requirement for a chronic oral study (83-1(a)) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

CP8349.MMO. February 21, 1996 E1 SBG.

Tox Chem No. 214 File Last Updated ______ Current Date ______

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	CORE Grade Doc. No.
Chronic Feeding (Gavage) Study in Rats (GLN 83-1) International Res. & Development Corp. #656-003 June 14, 1995	Chloropicrin (99%) Technical	MRID # 43744301	Dosing (gavage) levels of 0; 0.1; 1.0 & 10 mg/kg NOEL = 0.1 mg/kg ~~LEL~~ LOEL = 1.0 mg/kg based on subcutaneous masses and reduced body weights in females and reduced BW. in males,	—	Acceptable

SBG, 2/22/96

4

DATA EVALUATION REPORT

CHLOROPICRIN

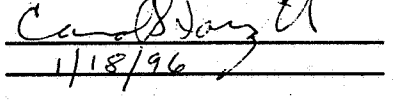
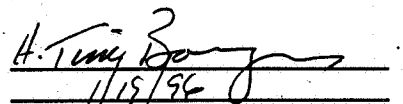
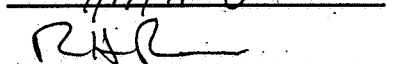
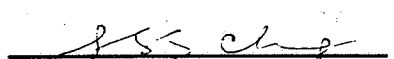
STUDY TYPE: CHRONIC ORAL TOXICITY [GAVAGE] - RAT (83-1(a))

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 95-17

Primary Reviewer: Carol S. Forsyth, Ph.D.	Signature: Date:	 1/18/96
Secondary Reviewers: H.Tim Borges, M.S.(ASCP), Ph.D., D.A.B.T.	Signature: Date:	 1/18/96
Robert H. Ross, M.S., Group Leader	Signature: Date:	 1/18/96
Quality Assurance: Susan Chang, M.S.	Signature: Date:	 1-18-96

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

S. Gross
EPA Reviewer: ~~E. Budd, M.A.~~ Date 2/14/96
Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. Date 2/21/96
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [gavage] - rat
OPPTS 870.4100 [§83-1(a)]

DP BARCODE: D218349
P.C. CODE: 081501

SUBMISSION CODE: S491939
TOX. CHEM. NO.: 214

TEST MATERIAL (PURITY): Chloropicrin (99%)

SYNONYMS: trichloronitromethane

CITATION: Slauter, R.W. (1995) Two year oral (gavage) chronic toxicity study of chloropicrin in rats. International Research and Development Corporation (IRDC), Mattawan, MI. Laboratory Project Identification 656-003, June 19, 1995. MRID 43744301. Unpublished.

SPONSOR: The Chloropicrin Manufacturers Task Force, c/o Stephen Wilhelm, Niklor Chemical Company, 2060 E. 228th Street, Long Beach, CA 90810.

EXECUTIVE SUMMARY: In a two-year chronic toxicity study (MRID 43744301), chloropicrin (99%a.i.) was administered to groups of 30 male and 30 female Sprague-Dawley Crl: CD® BR, VAF/Plus rats by gavage at dose levels of 0, 0.1, 1.0, and 10 mg/kg/day for 104 weeks.

The only clinical toxicity observed was increased salivation immediately after dosing in males and females receiving 10 mg/kg/day. This finding was observed in 4-56% of each sex and persisted 15-30 minutes. At terminal sacrifice, survival rates in the 0, 0.1, 1.0, and 10 mg/kg/day groups were 53%, 43%, 37%, and 50%, respectively for males and 40%, 50%, 53%, and 30%, respectively for females. Body weights of males in the high-dose group were lower (not significant) than controls beginning at week 30 and continuing until the end of the study. Final body weights of the 1.0 and 10 mg/kg/day males were 88% of the control group mean (not statistically significant). Among females, the 10 mg/kg/day group had a significantly ($p \leq 0.05$) greater mean body weight as compared to controls at week 4 but no other differences were noted. There were no differences between treated and control groups of either sex for food consumption, urinalysis, hematology parameters, or organ weights at sacrifice. Several clinical chemistry values were sporadically significantly different from the control value but there were no dose- or treatment-related trends. At necropsy, there was a dose-related increase in the incidence of subcutaneous masses of the skin in females: 14, 19, 24 ($p \leq 0.01$), and 29 ($p \leq 0.01$) for the 0, 0.1, 1.0, and 10 mg/kg/day groups, respectively. Hyperkeratosis of the nonglandular stomach was seen in 7/30, 9/30, 11/30, and 20/30 ($p \leq 0.01$) males and in 6/30, 5/30, 11/30, and 24/30 ($p \leq 0.01$) females, respectively. Hyperplasia of the nonglandular stomach was observed in 3/30, 5/30, 4/30, and

6

18/30 ($p \leq 0.01$) males and in 6/30, 5/30, 6/30, and 14/30 ($p \leq 0.05$) females, respectively. The incidence of inflammation of the stomach was significantly ($p \leq 0.05$) increased in high-dose females (5/30 vs. 0/30 controls) but, while increased in a dose-related manner in males (1/30, 1/30, 2/30, and 6/30), did not reach statistical significance. Periportal hepatocyte vacuolation occurred in a significantly greater number of females in the 1 mg/kg/day ($p \leq 0.05$) and 10 mg/kg/day ($p \leq 0.01$) groups: 2/30, 6/30, 10/30, and 13/30. Hepatocyte vacuolation was significant in males only in the 0.1 mg/kg/day group (2/30, 8/30, 3/30, and 6/30). Females had a dose-related increase in the incidence of fibroadenoma of the mammary gland with statistical significance reached in the high-dose group ($p \leq 0.05$); incidence rates were 6/30, 9/30, 12/30, and 14/30 affected in the 0, 0.1, 1.0, and 10 mg/kg/day groups, respectively. High-dose females also had an increase ($p \leq 0.01$) in the rate of C-cell hyperplasia of the thyroid (23/30 vs. 13/30 controls). **The LOEL is 1.0 mg/kg/day, based on subcutaneous masses and hepatocyte periportal vacuolation in female rats and reduced body weights in males. The NOEL is 0.1 mg/kg/day.**

This chronic toxicity study in the rat is acceptable and does satisfy the guideline requirement for a chronic oral study (83-1(a)) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chloropicrin

   Description: clear liquid
   Lot/Batch #: 920130-1
   Purity: 99% a.i.
   Stability of compound: received March 6, 1992; shelf life/expiration date February 1996
   CAS #: 76-06-2
   Structure: $Cl_3C\text{-}NO_2$

2. Vehicle and/or positive control

   Corn oil was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

   Species: rat
   Strain: Sprague-Dawley Crl: CD® BR, VAF/Plus
   Age and weight at study initiation: 43 days; males: 139-166 g; females: 119-141 g
   Source: Charles River Laboratories, Portage, MI
   Housing: Animals were housed three per cage for the first two days of acclimation then housed individually for the duration of the study in wire-mesh stainless steel cages.
   Diet: Certified Rodent Chow® #5002 was available *ad libitum*.

Water: Water was available *ad libitum* via an automatic watering system.
Environmental conditions:
Temperature: 65-78°F
Humidity: 40-70%
Air changes: "regulated"
Photoperiod: 12-hour light/dark
Acclimation period: 13 days

011890

B. STUDY DESIGN

1. In life dates

start: June 22, 1992 end: June 20, 1994

2. Animal assignment

Animals were assigned to a control or one of three test groups using the Xybion block randomization procedure in which animals were stratified by body weight. Homogeneity of group variance by body weight was used as the criterion for acceptance. Prior to study initiation, five animals per sex were selected for clinical pathology testing and microbiological screening. Animal assignment and dose levels are listed in Table 1.

TABLE 1: DOSAGE LEVELS AND ANIMAL ASSIGNMENT

Test Group	Dosage Level (mg/kg/day)	Main Study 104 weeks	
		male	female
Control	0	30	30
Low (LDT)	0.1	30	30
Mid (MDT)	1.0	30	30
High (HDT)	10.0	30	30

Data taken from Table 4.2.2.-1, p. 13, MRID 43744301.

3. Dose selection rationale

Doses were selected from pilot dose-range finding studies conducted with chloropicrin. In a 13-week gavage study, deaths in 10% of males and 40% of females occurred at 30 mg/kg/day. Also at this dose, reduced body weight gain in males and acanthosis and hyperkeratosis of the nonglandular stomach in males and females

were observed. Based on these data, doses of 0.1, 1, and 10 mg/kg/day were chosen for the present study.

4. Dose solution preparation and analysis

Suspensions of test article were prepared at concentrations such that doses were administered at a volume of 2 mL/kg. Dosage calculations were based on a density of 1.6558 g/mL. The 1 mg/kg and 10 mg/kg dosing suspensions were prepared by weighing the appropriate amount of vehicle into a glass container. The test article was measured, added to the vehicle, and mixed using a magnetic stir bar and stir plate for at least 30 minutes. The 0.1 mg/kg dosing solution was prepared by dilution of an appropriate amount of the 1 mg/kg solution with a measured amount of vehicle. Suspensions were prepared weekly and stored refrigerated until used. Samples from each concentration prepared for study week one were stored refrigerated for 14 days then analyzed for stability. Samples of each suspension from the first four weeks of study and every four weeks thereafter were analyzed for test article concentration. Homogeneity was not tested.

Results -
Stability Analysis: After refrigerated storage for 14 days, dosing solutions were 103 to 111% of the initial measured test article concentration.

Concentration Analysis: All dosing solutions were within 86 to 115% of nominal with the exception of the low- and mid-dose solutions prepared for study week 48 which began on May 17, 1993. These solutions, prepared on May 13, 1993, contained 121% and 120% of target, respectively. Replacement batches were made on May 19, 1993 and shown to contain 103% and 103% of nominal, respectively.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body weight and food consumption values, hematological, biochemical and urological parameters, and absolute and relative organ weights were analyzed using Bartlett's test for homogeneity of variances, 1-way analysis of variance, and the appropriate t-test. Dunnett's multiple comparison tables or pairwise comparisons with a Bonferroni correction were used to determine the significance of differences. Nonparametric analyses were conducted as appropriate by transforming the data into ranks prior to analysis. Mammary gland fibroadenoma data were analyzed by procedures including life table tests, the Hoel-Walburg "incidental tumor" tests, Fisher's exact tests, and Cochran-Armitage trend tests. Levels of significance were set at $p \leq 0.05$ and $p \leq 0.01$.

9

C. METHODS

1. Observations

Animals were observed twice daily for signs of toxicity and morbidity/mortality. Detailed observations of appearance and condition, behavior and activity, excretory function, respiration, orifices, eyes, and palpable masses were conducted once weekly. Transient, frequently recurring observations were recorded daily during study weeks 2 and 3, five days during week 4, and weekly thereafter.

2 Body weight

Individual animal body weights were recorded prior to study initiation, weekly during the first 14 weeks of study, and then once every four weeks until sacrifice.

3. Food consumption

Individual food consumption was measured weekly during the first 14 weeks of study and then once every four weeks until sacrifice.

4. Ophthalmoscopic examination

All animals were given an ophthalmoscopic examination of the cornea, conjunctiva, sclera, iris, and fundus once during the acclimation period and prior to sacrifice.

5. A pretest microbiological screen was conducted on blood samples from 5 animals/sex to determine the presence of the following: pneumonia virus of mice, reovirus type 3, rat coronavirus/sialodacryoadenitis virus, encephalomyelitis virus, Sendai virus, lymphocytic choriomeningitis virus, Kilham rat virus, Toolan's H-1 virus, and *Mycoplasma pulmonis*.

Blood was collected for hematology and clinical chemistry analysis from 10 randomly selected animals/sex/group at 3, 6, 12, 18, and 24 months of study. The same animals were used at each interval when possible. Blood was collected from the orbital sinus following an overnight fast during which water was available. The CHECKED (X) parameters were examined:

10

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophoresis
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected during the overnight fasting from the animals used for blood collection. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen
		X	Leukocytes

* Required for chronic studies

7. Sacrifice and pathology

All surviving animals and those sacrificed moribund were euthanitized by carbon dioxide inhalation. All animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The [XX] organs, in addition, were weighed. A full complement of organs and tissues was processed for microscopic examination from all animals in the 0 mg/kg/day and 10 mg/kg/day groups and from any animal dying or killed during the study. In addition, sections were prepared from the liver, kidney, lung, nonglandular stomach, and gross lesions or masses from all animals, and the mammary gland of all females in the 0.1 mg/kg/day and 1.0 mg/kg/day groups.

12

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	X	Adrenal gland*
X	Rectum*	X	Urinary bladder*		Lacrimal gland
XX	Liver*+	XX	Testes*+	X	Mammary gland*
X	Pancreas*	X	Epididymides	X	Parathyroids*
		X	Prostate	X	Thyroids*
	RESPIRATORY	X	Seminal vesicle		
X	Trachea*	X	Ovaries*+		OTHER
X	Lung*	X	Uterus*	X	Bone*
X	Nose			X	Skeletal muscle*
	Pharynx			X	Skin*
	Larynx			X	All gross lesions and masses*

*Required for chronic studies based on Subdivision F Guidelines.
+Organ weight required in chronic studies.

II. RESULTS

A. Observations

1. Toxicity

Immediately after dosing, increased salivation was seen in males and females receiving 10 mg/kg/day. This finding was observed in 4-56% of each sex and persisted 15-30 minutes. Clinical signs not considered treatment related in both male and female rats included hair loss, malocclusion, labored breathing, material around eye or area around eye red, decreased defecation, staining of the body surface, and decubital ulcers. These signs were observed equally in treated and control groups. Table 2 shows that the number of animals appearing normal or not showing any adverse clinical signs decreased over time throughout the study.

TABLE 2: ANIMALS WITH NO VISIBLE ABNORMALITIES N (%)[a]

Interval	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	10 mg/kg/day
Males				
Weeks 1-13	30 (100)	30 (100)	30 (100)	30 (100)
Weeks 53-65	22 (73.3)	20 (66.7)	18 (66.7)	19 (63.3)
Weeks 92-104	8 (36.4)	3 (15.8)	7 (33.3)	6 (30)
Females				
Weeks 1-13	30 (100)	30 (100)	30 (100)	30 (100)
Weeks 53-65	20 (66.7)	21 (75)	22 (73.3)	20 (71)
Weeks 92-104	4 (23.5)	10 (45.5)	8 (33.3)	2 (11.8)

Data taken from Table 2, pp. 60-87, and Appendix C, pp. 235-243, MRID 43744301.
[a]Percentage based on number of animals alive at beginning of interval.

2. Mortality

At terminal sacrifice, survival rates in the 0, 0.1, 1.0, and 10 mg/kg/day groups were 53%, 43%, 37%, and 50%, respectively for males and 40%, 50%, 53%, and 30%, respectively for females. Because the guidelines for a chronic oral study require 25% survival-at-termination, the high-dose females were terminated during study week 103.

B. Body weight

Body weights at selected time points during the study are presented in Table 3. For males, no statistically significant differences occurred between the treated and control groups at any time. However, body weights of males in the high-dose group were lower than controls beginning at week 30 and continuing until the end of the study. Final body weights of the 1.0 and 10 mg/kg/day males were only 88% of the control group mean. Among females, the 10 mg/kg/day group had a significantly ($p \leq 0.05$) greater mean body weight as compared to controls at week 4. When this group was terminated at week 103, final body weights were 102% of the control mean.

14

TABLE 3: BODY WEIGHTS (G) OF RATS GIVEN CHLOROPICRIN BY GAVAGE FOR 104 WEEKS				
Week of Study	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	10 mg/kg/day
Males				
0	174 ± 8.2	173 ± 8.2	174 ± 9.0	174 ± 8.3
2	267 ± 18.6	269 ± 15.3	268 ± 17.4	267 ± 16.2
4	336 ± 24.3	338 ± 23.4	337 ± 25.4	335 ± 26.3
8	426 ± 38.7	433 ± 33.9	430 ± 34.4	428 ± 36.6
12	482 ± 45.5	491 ± 41.9	490 ± 43.2	481 ± 40.1
30	576 ± 59.0	590 ± 62.2	588 ± 64.2	569 ± 55.6
50	661 ± 78.6	670 ± 75.0	670 ± 88.7	635 ± 72.9
70	709 ± 91.5	670 ± 90.8	708 ± 98.0	680 ± 80.5
90	687 ± 121.3	671 ± 91.0	684 ± 83.3	622 ± 100.7
104	697 ± 139.9	651 ± 102.8	616 ± 66.8	616 ± 110.1
Females				
0	140 ± 6.0	141 ± 6.6	141 ± 7.8	141 ± 6.5
2	183 ± 9.9	184 ± 11.2	184 ± 12.9	186 ± 12.4
4	212 ± 13.1	213 ± 13.1	214 ± 16.2	222 ± 14.5*
8	253 ± 17.1	252 ± 15.9	251 ± 19.6	260 ± 21.3
12	277 ± 19.0	278 ± 20.2	276 ± 22.5	287 ± 27.0
30	327 ± 32.4	327 ± 33.6	326 ± 30.9	340 ± 44.6
50	393 ± 49.1	390 ± 51.7	385 ± 45.7	406 ± 59.7
70	436 ± 70.4	450 ± 65.7	443 ± 56.8	448 ± 79.2
90	451 ± 108.7	455 ± 83.5	444 ± 97.1	451 ± 87.2
104	472 ± 116.7	461 ± 90.9	454 ± 80.8	--

Data taken from Table 3, pp. 88-91, MRID 43744301.
*Significantly different from control, $p \leq 0.05$.

15

C. Food consumption

Selected mean food consumption values are listed in Table 4. Males in the 10 mg/kg/day group ate significantly ($p \leq 0.05$) less than controls during weeks 18 and 98, but all other values were comparable to controls. No differences occurred for males in the 0.1 or 1.0 mg/kg/day groups as compared to controls. Food consumption in all treated female groups was sporadically greater than the control throughout the study. Statistical significance was reached in the 0.1 mg/kg/day group at weeks 26 and 58 ($p \leq 0.01$), in the 1.0 mg/kg/day group at weeks 13 and 54 ($p \leq 0.05$), and in the 10 mg/kg/day group at weeks 26, 54, and 58 ($p \leq 0.01$).

TABLE 4: FOOD CONSUMPTION (G/RAT/DAY) BY RATS GIVEN CHLOROPICRIN BY GAVAGE FOR 104 WEEKS

Week of Study	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	10 mg/kg/day
Males				
1	22.0 ± 1.68	21.8 ± 1.72	22.4 ± 1.61	22.2 ± 1.47
2	22.5 ± 2.45	23.0 ± 1.73	23.3 ± 1.95	22.5 ± 1.9
4	24.5 ± 2.41	24.9 ± 2.03	24.6 ± 2.13	24.3 ± 2.37
8	24.5 ± 2.86	25.1 ± 2.36	24.7 ± 2.07	24.2 ± 2.70
12	24.4 ± 2.75	24.8 ± 2.88	25.3 ± 2.34	23.5 ± 2.42
30	24.9 ± 2.55	24.4 ± 2.55	25.0 ± 2.39	24.5 ± 2.35
50	23.2 ± 1.80	23.3 ± 2.26	23.0 ± 2.35	22.0 ± 2.86
70	22.8 ± 2.88	21.8 ± 3.77	23.0 ± 3.72	23.3 ± 3.72
90	22.3 ± 6.22	23.3 ± 4.22	22.1 ± 3.37	20.5 ± 3.53
104	19.8 ± 3.27	18.8 ± 3.11	17.3 ± 2.53	18.9 ± 4.60
Females				
1	16.1 ± 1.19	16.4 ± 1.38	16.8 ± 1.67	16.5 ± 1.73
2	16.4 ± 1.23	16.9 ± 1.61	17.1 ± 1.83	16.7 ± 2.58
4	18.0 ± 1.70	18.5 ± 1.52	18.6 ± 2.04	18.3 ± 2.06
8	17.6 ± 1.94	18.5 ± 1.42	18.3 ± 1.76	18.5 ± 1.96
12	17.4 ± 1.62	18.3 ± 1.30	18.4 ± 1.93	17.4 ± 1.70
30	19.1 ± 2.39	19.3 ± 2.11	19.4 ± 2.19	19.0 ± 2.80

TABLE 4. Continued

50	18.8 ± 1.96	18.6 ± 2.07	18.6 ± 3.06	19.1 ± 2.79
70	18.4 ± 5.12	20.5 ± 2.49	20.0 ± 3.09	19.2 ± 3.48
90	17.4 ± 4.93	20.1 ± 5.31	17.2 ± 5.02	15.5 ± 7.56
104	15.4 ± 4.79	15.7 ± 4.69	15.5 ± 5.87	--

Data taken from Table 4, pp. 92-95, MRID 43744301.

D. Ophthalmoscopic examination

The eyes of all animals appeared normal at pretest. At termination, findings such as conjunctivitis, keratitis, cataracts, and phthisis bulbi were observed in all treated and control groups of both sexes. No dose- or treatment-related trend was apparent.

E. Blood work

1. Hematology

The results from the pretest viral screen were negative for all animals tested.

During the study, no statistically significant differences in hematological parameters were observed between treated and control groups of either sex.

2. Clinical chemistry

Several clinical chemistry values were sporadically different from the control values throughout the study. These are listed in Table 5. With the exception of the value for aspartate aminotransferase in the 10 mg/kg/day males, significant differences between treated and control groups occurred at only one of the five time points tested during the study. All values for the male and female treated groups were within 35% of their respective control group measurement.

17

TABLE 5: SELECTED CLINICAL CHEMISTRY PARAMETERS

Parameter	Month of Study[a]	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	10 mg/kg/day
Males					
Aspartate Aminotransferase (U/L)	3 6 12	105 100 78	99 (94)[b] 85 (85) 75 (96)	114 (109) 92 (92) 86 (110)	83 (79)* 74 (74)** 55 (71)**
Alanine Aminotransferase (U/L)	3	26	33 (127)*	35 (135)**	29 (112)
Urea Nitrogen (mg/dL)	3	14	13 (93)	17 (121)**	15 (107)
Females					
Calcium (mg/dL)	24	9.9	10.1 (102)	10.1 (102)	11.2 (113)**
Phosphorus (mg/dL)	24	4.3	4.3 (100)	4.2 (98)	5.3 (123)**
Total Bilirubin (mg/dL)	24	0.4	0.4 (100)	0.4 (100)	0.3 (75)**
Aspartate Aminotransferase (U/L)	3	109	100 (92)	102 (94)	86 (79)*
Creatinine (mg/dL)	24	0.4	0.5 (125)	0.4 (100)	0.5 (125)*

Data taken from Table 6, pp. 104-113, MRID 43744301.
[a]Five blood samples were collected throughout the study for clinical chemistry evaluation.
[b]Numbers in parentheses are per cent of control.
*Significantly different from control, $p \leq 0.05$.
**Significantly different from control, $p \leq 0.01$.

F. Urinalysis

No statistically significant differences in urinalysis parameters were observed at any time point for either sex between treated and control groups.

G. Sacrifice and pathology

1. Organ weight

There were no statistically significant differences in absolute or relative organ weights from treated groups of either sex as compared to controls.

2. Gross pathology

At necropsy, there was a dose-related increase in the incidence of subcutaneous masses of the skin in females. The incidence rates were 14, 19, 24 ($p \leq 0.01$), and 29 ($p \leq 0.01$) for the 0, 0.1, 1.0, and 10 mg/kg/day groups, respectively; many of the masses were shown to be mammary fibroadenomas (see below). Other observations in both sexes of treated and control rats included corneal opacity, enlarged adrenal glands, swelling and ulceration of the feet, red or tan foci in the livers, red discoloration of the lungs, red foci and enlarged pituitaries, enlarged spleens, and absent/broken/malocclusion/overgrown teeth. Males also had granular surface and tan discoloration of the kidneys, enlarged parathyroids, and small and/or soft testes. In females, mammary changes and cysts in the ovaries and uteri were also observed.

3. Microscopic pathology

a) Non-neoplastic — The incidence rates of selected histopathological observations are given in Table 6. Hyperkeratosis and hyperplasia of the nonglandular stomach was observed in a significantly greater number of males ($p \leq 0.01$) and females ($p \leq 0.01$ and $p \leq 0.05$, respectively) in the 10 mg/kg/day group as compared to controls. The incidence of inflammation of the stomach was significantly ($p \leq 0.05$) increased in high-dose females but, while increased in a dose-related manner in males, did not reach statistical significance. Periportal hepatocyte vacuolation occurred in a significantly greater number of females in the 1 mg/kg/day ($p \leq 0.05$) and 10 mg/kg/day ($p \leq 0.01$) groups. In these two groups, liver sections stained positive with Oil Red O in 1 and 5 animals, respectively. Hepatocyte vacuolation was significant in males only in the 0.1 mg/kg/day group.

Females had a dose-related increase in the incidence of fibroadenoma of the mammary gland with significantly ($p \leq 0.05$) greater numbers affected in the 10 mg/kg/day group. High-dose females also had an increase ($p \leq 0.01$) in the rate of C-cell hyperplasia of the thyroid.

TABLE 6: MICROSCOPIC FINDINGS IN RATS GIVEN CHLOROPICRIN BY GAVAGE FOR 104 WEEKS [incidence (%)]

Finding	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	10 mg/kg/day
Males				
Stomach, nonglandular				
hyperkeratosis	7 (23)	9 (30)	11 (37)	20** (67)
hyperplasia	3 (10)	5 (17)	4 (13)	18** (60)
inflammation	1 (3)	1 (3)	2 (7)	6 (20)
Liver, periportal vacuolation	2 (7)	8* (27)	3 (10)	6 (20)
Females				
Stomach, nonglandular				
hyperkeratosis	6 (20)	5 (17)	11 (37)	24** (80)
hyperplasia	6 (20)	5 (17)	6 (20)	14* (47)
inflammation	0	0	2 (7)	5* (17)
Liver, periportal vacuolation	2 (7)	6 (20)	10* (33)	13** (43)
Mammary Gland, fibroadenoma	6 (20)	9 (30)	12 (40)	14* (47)
Thyroid, C-cell hyperplasia	13 (43)	5* (17)	10 (33)	23** (77)

Data taken from Table 10, pp. 147-212, MRID 43744301.
*Significantly different from control, $p \leq 0.05$; calculated by reviewer using the Fisher Exact Test.
**Significantly different from control, $p \leq 0.01$; calculated by reviewer using the Fisher Exact Test.

b) Neoplastic – There were no dose- or treatment-related neoplastic lesions in males or females. The dose-related increase in females of mammary fibroadenoma was not accompanied by other proliferative mammary lesions such as adenocarcinoma or squamous cell carcinoma.

30

III. DISCUSSION

A. Male and female Sprague-Dawley (Crl: CD® BR VAF/Plus) rats were given chloropicrin by gavage at doses of 0, 0.1, 1.0, or 10 mg/kg/day for 104 weeks. Survival rates were not different between treated and control groups. However, the high-dose females were approaching the guideline requirement of 25% survival-at-termination so were sacrificed during week 103. Transient, post-dosing salivation was observed in 4-56% of males and females receiving 10 mg/kg/day and was most likely due to the irritating nature of the test article rather than a systemic effect. Other clinical signs reported throughout the study including hair loss, malocclusion, labored breathing, and decubital ulcers increased with frequency and are consistent with aging rats. At sacrifice, the ophthomology findings are also consistent with older animals. The lower final body weights of mid- and high-dose males as compared to the control were not statistically significant but are considered to be biologically significant. Final body weights of these groups were only 88% of the control value while food consumption was not affected. Therefore, the effect on body weights in males is most likely compound-related. No dose- or treatment-related trends were apparent in the hematology or clinical chemistry findings. Significant differences found in several clinical chemistry values are not considered biologically significant since the difference from the controls was small and the results were not consistent throughout the study.

At necropsy, no significant differences were found in absolute or relative organ weights from treated groups of either sex as compared to controls. There was a dose-related increase in the number of subcutaneous masses of the skin in females. The incidence rates were significantly ($p \leq 0.01$) greater in the 1.0 and 10 mg/kg/day groups as compared to the control group (24/30 and 29/30 vs. 14/30, respectively). Many of these masses were shown to be fibroadenomas of the mammary gland. The proportion of females having fibroadenoma in the 10 mg/kg/day is 47% which is significantly ($p \leq 0.05$) greater than the controls for the present study. This rate is within the historical control incidence, 6.7-55%, provided with the study. However, because the incidence of fibroadenoma among treated females in the present study is dose-related and all treated groups are above the control level, the lesion is considered compound-related.

At 10 mg/kg/day, hyperkeratosis and hyperplasia of the nonglandular portion of the stomach was observed in a significantly greater number of males ($p \leq 0.01$) and females ($p \leq 0.01$ and $p \leq 0.05$, respectively) as compared to controls. The incidence of inflammation of the stomach was significantly ($p \leq 0.05$) increased in high-dose females and was increased in a dose-related manner in males. Although the incidence of inflammation of the stomach did not reach statistical significance in males, the effect is probably treatment-related based on the results in females. Hyperkeratosis, hyperplasia, and inflammation of the stomach are probably due to the irritating nature of the test article.

21

Periportal hepatocyte vacuolation occurred in a significantly greater number of females in the 1 mg/kg/day ($p \leq 0.05$) and 10 mg/kg/day ($p \leq 0.01$) groups. In these two groups, liver sections stained positive with Oil Red O in 1 and 5 animals, respectively, indicating that vacuolation was due, in part, to fatty accumulation. Hepatocyte vacuolation was significant in males only in the 0.1 mg/kg/day group.

Therefore, under the conditions of this study the LOEL is 1.0 mg/kg/day based on subcutaneous masses and hepatocyte periportal vacuolation in female rats and reduced body weights in male rats. The NOEL is 0.1 mg/kg/day.

B. Study deficiencies

There were no deficiencies in the conduct of the study.

83-1 Chronic Feeding in the Rodent and Nonrodent

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration in rodents minimum 12 month nonfood use, 24 months food use; in nonrodents minimum 12 months[1].
4. ___ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1,000 mg/kg).
5. • ✓ Doses tested include a NOEL.
6. • ✓ Analysis for test material stability, ~~homogeneity~~ 83-3 and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pertest and at term) control and high dose.
11. ✓ Clinical pathology data for all nonrodents and at least 10 rodents/group consisting of 12, 13 & 14.
13. ✓ Hematology at 6 month intervals consisting of at least;
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
14. ✓ Clinical chemistry at 6 month intervals consisting of at least;
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ✓ Creatinine kinase
    - ✓ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
15. ✓ Urinalysis at 6 month intervals consisting of at least;
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume
16. ✓ Individual necropsy of all animals.
17. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

Criteria marked with a • are supplemental and may not be required for every study.

✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung†	✓ ovaries†
✓ duodenum	✓ lymph nodes	___ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart†	✓ spleen†	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	✓ salivary glands
✓ ileum	✓ adrenals†	✓ thymus
✓ trachea	✓ uterus	✓ urinary bladder

† organs to be weighed

Six month dog studies may be acceptable. (?)

Criteria marked with a • are supplemental and may not be required for every study.